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EXCITOTOXIC AMINO ACIDS AND NEUROPSYCHIATRIC DISORDERS

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INTRODUCTION

A wealth of new information pertaining to excitatory amino acids (EAA) has been generated over the past two decades. The prototypic EAA, glutamate (Glu) and aspartate (Asp), which are abundantly present in the mammalian central nervous system, have become recognized as Jekyll and Hyde molecules that serve vitally important metabolic and neurotransmitter functions while simultaneously harboring treacherous neurotoxic potential. The neurotoxicity of Glu and Asp, although first described 30 years ago, was relatively ignored until studies in the early 1970s linked the phenomenon to an excitatory mechanism. In subsequent years, mechanisms underlying excitotoxic phenomena have been further elucidated, several EAA receptor subtypes have been delineated, drugs with anti-excitotoxic actions have been identified, and evidence for the potential complicity of excitotoxins in neurodegenerative disorders has begun to unfold. A specific subtype of EAA receptor, the N-methyl-D-aspartate (NMDA) receptor, has become a primary focus of attention because of evidence implicating it in a wide range of both neurophysiological and pathological processes. Evidence for the involvement of other EAA receptor subtypes in human neuropathological syndromes has also begun to appear and there is basis for believing that central neurons may be particularly vulnerable to excitotoxic degeneration during certain periods of development and in old age. Here I review highlights of research de-

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velopments in excitotoxicology with an emphasis on the potential clinical implications of this line of research.

THE EXCITOTOXIC CONCEPT

Historical Perspective

Three decades ago, Curtis, Watkins and colleagues (1, 2) used newly developed microelectrophoretic techniques to examine the membrane-depolarizing properties of Glu and related compounds and characterize the structural requirements for interaction with an apparent excitatory amino acid (EAA) receptor. However, the myriad metabolic involvements of Glu, its ability to excite neurons throughout the CNS and the lack of any known mechanism for terminating its excitatory action led neuroscientists of the 1960s to reject Glu as a transmitter candidate. This view prevailed for two decades before yielding slowly to evidence suggesting that Glu satisfies the criteria for a transmitter. Finally, in the 1980s, Glu has gained widespread acceptance as the front-running transmitter candidate at the majority of excitatory synapses in the mammalian CNS.

Because Glu is found in very high concentration in the CNS and serves a number of important metabolic functions, early reports that systemic administration of Glu to infant mice destroys neurons in the retina (3, 4), or in certain regions of brain (5), were met with disbelief. However, CNS damage following either oral or subcutaneous administration was readily reproduced in a number of animal species, including primates (6–8). In addition, it was shown (9) that specific Glu analogs (Figure 1) known to share the neuroexcitatory properties of Glu mimic its neurotoxic effects, that these analogs have a parallel order of potencies for their excitatory and toxic actions, and that analogs lacking excitatory activity also lack neurotoxicity. Moreover, ultrastructural studies (6, 10) localized the apparent site of toxic action to postsynaptic dendrosomal membranes where Glu excitatory synaptic receptors are located. These and related observations gave rise to the excitotoxic concept that Glu destroys neurons by excessive activation of excitatory receptors on the dendrosomal surfaces of neurons.

Excitotoxin Receptors and Anti-Excitotoxic Drugs

Of major importance for developing better insight into the physiological and pathological properties of EAA transmitter systems was the identification of EAA receptor subtypes differentially sensitive to specific agonists [N-methyl-D-aspartate (NMDA), quisqualic acid, kainic acid (KA)], and the discovery of antagonists that block the excitatory actions of EAA agonists at such receptors (2, 11–13). Shortly after they were first identified, EAA antagonists were shown to protect neurons in the in vivo mouse hypothalamus against the

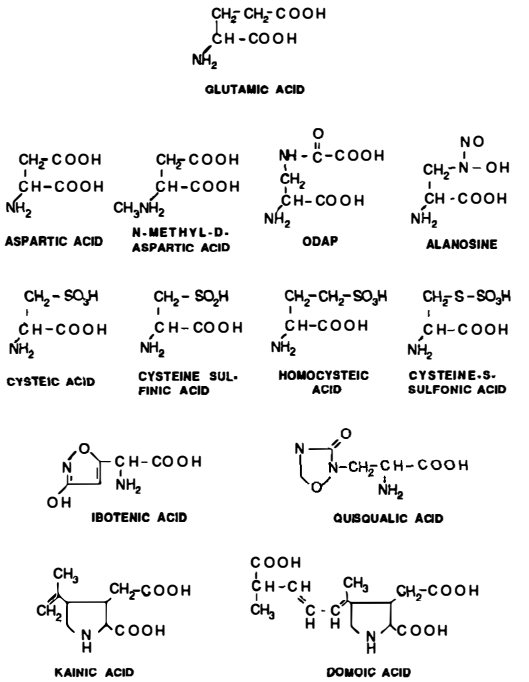

Figure 1 Acidic amino acids that mimic both the excitatory and neurotoxic properties of glutamate. ODAP = β-N-oxalylamino-L-alanine (also abbreviated BOAA).

neurotoxic actions of Glu or its more potent analog, NMDA (14, 15). Similar anti-excitotoxic effects were subsequently shown either in vivo (16) or in vitro (17) for these or other individual agents with known blocking action against the excitatory effects of EAA agonists. In further confirmation of the excitotoxic hypothesis, many EAA antagonist candidates have now been systematically screened in in vitro preparations and found to have anti-excitotoxic activities corresponding in potency and receptor specificity to their known anti-excitatory activities (18–20).

Evidence implicating excitotoxins in neurodegenerative disorders (see below), stimulated interest in the possible value of EAA antagonists as neuroprotective agents in clinical neurology. In Table 1, the results of several EAA agonist/antagonist studies (18–20) are summarized. The first generation of EAA antagonists identified (13) were competitive NMDA antagonists that compete with NMDA agonists for binding at NMDA receptors. Agents in this class, although possessing moderately potent antagonist properties, are of uncertain value for clinical applications as they do not readily penetrate blood-brain barriers. The most powerful anti-excitotoxic drugs identified thus

far are noncompetitive NMDA antagonists that act at phencyclidine receptors to block both the excitatory (21, 22) and toxic (18, 19) actions of NMDA. MK-801, a drug developed by Merck, Sharp and Dohme, is the most potent known compound in this category. Because these compounds do penetrate blood-brain barriers, they are of interest as potential therapeutic agents. Certain currently marketed drugs, including dextromethorphan (23) and several anti-Parkinsonian agents (24), are moderately potent noncompetitive NMDA antagonists. Mixed antagonists, such as kynurenic acid and cis-2,3-piperidine dicarboxylate, block the excitotoxic effects of both NMDA and non-NMDA agonists but are of limited interest because of their low potency and inability to penetrate blood-brain barriers. CNQX, a recently described quinoxalinedione (25), has the important distinction of being the first agent found to block the excitatory (25) and excitotoxic (26) actions of non-NMDA agonists more powerfully than it blocks those of NMDA. Certain thiobarbiturates penetrate blood-brain barriers and are moderately potent against both NMDA and non-NMDA agonists (27, 28).

Table 1 Potencies of antagonists in blocking NMDA or KA toxicity[a]

Potential antagonist	vs NMDA	vs KA
Competitive NMDA antagonists		
D-2-amino-5-phosphonopentanoate (AP 5)	25	>1000
D-2-amino-7-phosphonoheptanoate (AP 7)	75	>1000
D-Alpha-aminoadipate	200	>1000
Non-competitive NMDA antagonists		
MK-801	0.1	>1000
Phencyclidine	0.5	>1000
Ketamine	5	>1000
(±) Cyclazocine	5	>1000
(+) SKF 10,047	10	>1000
Dextromethorphan	50	>1000
Anti-Parkinsonian agents		
Procyclidine (Kemadrin)	15	>1000
Ethopropazine (Parsidol)	25	>1000
Mixed EAA antagonists		
CNQX	200	50
Kynurenic acid	300	750
(±)-Cis-2,3-piperidine dicarboxylate	1000	>1000
Barbiturates		
Thiamylal	50	250
Thiopental	200	400

[a]All tests were conducted in the isolated chick embryo retina by methods previously described (18). Compounds were rated according to the minimal concentration (μM) required to provide total protection against the excitotoxic effects of NMDA (80 μM) or KA (25 μM). Antagonists were tested over a range of concentrations from 1000 μM downward until a minimal effective concentration was established.

Acute versus Delayed Excitotoxic Cell Death

In vitro ion substitution experiments have provided evidence for more than one mechanism by which excitotoxin-induced neuronal degeneration can occur. In hippocampal cell cultures (29) or in the isolated chick retina (30), neurons degenerate very rapidly when exposed continuously for 30 min to a toxic concentration of Glu or any of its excitotoxic analogs. In either of these preparations, this acute toxic reaction is abolished by the removal of Na^+ or Cl^- from the incubation medium, but is not prevented or diminished by the removal of CA^{++} (29–31). However, Choi (32, 33) has described a slow degenerative process triggered by brief (5 min) exposure of cultured neurons to Glu, a process facilitated by the presence of Ca^{++} in the incubation medium. Evidence for Ca^{++}-dependency of EAA neurotoxicity has also been described in cerebellar slices (34). Thus, there is basis for believing that excitotoxins can destroy neurons by either an acute fulminating process which is Na^+- and Cl^-- (but not Ca^{++}) dependent, or by a slow process which is Ca^{++}-dependent. In the latter case, it is noteworthy that excitotoxic neuronal degeneration occurs even though the duration of exposure to an abnormal concentration of excitotoxin is only 1/6 as long as in the former case. A better understanding of the delayed type of mechanism is needed because many human neurological disorders entail the slow degeneration of neurons by a subacute or chronic mechanism. If excitotoxins play a role in such degenerative processes, to have a wide time window for therapeutic intervention would be highly advantageous.

Special Features of NMDA Receptors

At least three types of EAA receptor (NMDA, Quis, KA) are capable of mediating excitotoxic events. The most studied of these and reportedly the most abundant and widely distributed in the mammalian CNS (35), is the NMDA receptor. Several features distinguish the NMDA receptor from other subtypes of EAA receptor. This receptor is linked to a Na^+/Ca^{++} ion channel with a much higher Ca^{++} conductance than ion channels associated with other EAA receptor sub types (36) and the NMDA ion channel is subject to a voltage-dependent Mg^{++} blockade (37). The NMDA receptor is closely associated with a strychnine-insensitive glycine receptor (38), which facilitates opening of the NMDA ion channel, and with PCP receptors (21, 22), which are believed to be positioned within this channel (39) and permit PCP agonists to perform an open-channel block. In addition, there is evidence that Zn^{++}, acting at a separate site near the mouth of the NMDA ion channel, acts as an inhibitory modulator of channel function (40, 41). Thus, as Figure 2 illustrates, the NMDA receptor system is a remarkably complex entity, the normal function of which depends on a dynamic equilibrium among multiple facilitative and inhibitory factors. It follows that a pathological process

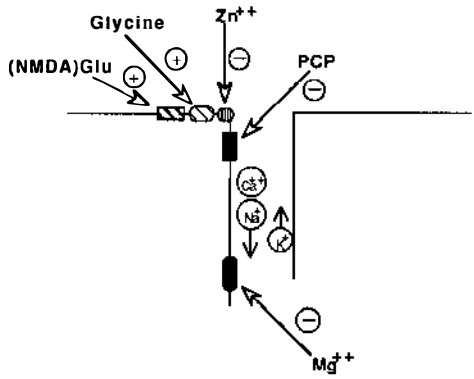

Figure 2 A schematic depiction of the various components comprising the NMDA receptor-ionophore complex. Recent evidence suggests that endogenous transmitter (e.g. Glu or Asp) released from presynaptic axon terminals activates NMDA receptors on postsynaptic dendrosomal membranes, which results in opening of a Na^+/Ca^{++} ion channel. Glycine, acting at strychnine insensitive receptors that are colocalized with NMDA receptors, facilitates opening of this channel, whereas PCP, Zn^{++} and Mg^{++}, each acting at a separate site and presumably by separate mechanisms, are antagonists of channel function.

affecting any given factor might create an imbalance rendering the entire system malfunctional. For example, increased efficacy of Glu or glycine or decreased efficacy of Zn^{++}, Mg^{++}, or the endogenous PCP ligand at their respective recognition sites would render the NMDA receptor system hyperfunctional and prone to an expression of excitotoxicity.

EXCITOTOXINS AND NEURODEGENERATIVE DISORDERS

Given their presence in the CNS in high concentrations and their intrinsic excitotoxic potential, EAA have long been suspected of causing neurodegenerative disorders. Over the past decade, evidence has begun to accumulate supporting such suspicion, as well as the companion suspicion that, in many cases, NMDA-type receptors may be major participants in the neurodegenerative process. Neurodegenerative conditions in which EAA and an excitotoxic mechanism may be involved are grouped into several categories and briefly discussed below.

Food Excitotoxins

GLU AND NEUROENDOCRINOPATHIES Glu, in the form of its sodium salt (monosodium glutamate, MSG) is one of the world's most widely and heavily used food additives. The Food and Drug Administration still places no restrictions on the food additive use of MSG, despite evidence that doses in the range of those sometimes fed unwittingly (as a food additive) to human

young, destroy CNS neurons when administered orally to immature animals. Neurons most vulnerable to destruction by orally administered MSG are those lying in certain brain regions that lack blood-brain barriers, e.g. neurons in the arcuate nucleus of the hypothalamus which regulate neuroendocrine function. Since destruction of hypothalamic neurons in immature animals results in a complex neuroendocrine deficiency syndrome, the question arises whether ingestion of MSG by human young contributes to the occurrence of neuroendocrinopathies in later life (for recent reviews of this issue, see 42, 43).

BOAA AND NEUROLATHYRISM Neurolathyrism is a crippling neurological disorder endemic in certain parts of the world where the legume, *Lathyrus sativus*, is ingested in excess during periods of famine. The poisonous ingredient in *L. sativus* is believed to be β-N-oxalylamino-L-alanine (BOAA, also sometimes abbreviated ODAP), an acidic amino acid with powerful excitotoxic properties (44–47). Spencer and coworkers (46) have recently demonstrated that the paralytic symptoms of neurolathyrism can be reproduced in monkeys maintained chronically on a diet enriched in BOAA. The neurotoxic effects of BOAA in tissue culture are blocked by broad-spectrum EAA antagonists (47, 48). Evidence linking exogenous excitotoxins to a paralytic disease such as neurolathyrism is of considerable interest; if chronic exposure to exogenous excitotoxins can cause neuronal degeneration that develops gradually over a period of months or years, it may be possible for endogenous excitotoxins to cause neuronal degeneration on a similarly chronic basis (e.g. in Huntington's chorea, Alzheimer's dementia, or parkinsonism). Moreover, should research on neurolathyrism reveal a mechanism by which ingested exogenous excitotoxins can cause degeneration of neurons in parts of the CNS thought to be inaccessible to such agents, it will be necessary to reevaluate the possibility that exogenous excitotoxins, including those used as food additives, might contribute (in concert with endogenous excitotoxins) to a variety of chronic neurodegenerative conditions.

BMAA AND ALS/PARKINSONISM/DEMENTIA COMPLEX Spencer and colleagues (49) have postulated that a neurological disease endemic to certain western Pacific islands, especially Guam, which has combined features of amyotrophic lateral sclerosis, parkinsonism and dementia, might be caused by exposure to the seeds of a cycad plant containing high concentrations of β-N-Methylamino-L-alanine (BMAA). It has been proposed that the gradual decrease in incidence of this disease in recent years can be attributed to a change in eating habits that has reduced the islanders' intake of BMAA (49). An excitotoxic mechanism has been suspected on the basis of in vitro studies showing that BMAA has neurotoxic properties that can be blocked by NMDA antagonists (47, 48). It is nevertheless puzzling that the BMAA molecule

lacks the omega acidic group which characterizes all other straight chain molecules with excitotoxic properties, including BOAA. After finding that BMAA displays stronger excitatory and neurotoxic activity in the presence of physiological concentrations of bicarbonate (50), Weiss & Choi proposed that bicarbonate interacts noncovalently with the positively charged beta-amino group of BMAA to produce a configuration appropriate for activation of Glu receptors. These studies provide an additional reminder that an investigation of mechanisms by which exogenous excitotoxins cause neuronal degeneration may eventually lead to a better understanding of mechanisms by which endogenous EAA, either alone or together with exogenous excitotoxins, can induce neurodegenerative processes.

DOMOATE POISONING In 1987, an outbreak of food poisoning in Canada affected 145 people, some of whom died and were found at autopsy to have disseminated acute lesions in the CNS (51). Some of the survivors apparently sustained permanent brain damage as they have continued to show signs of mental confusion and profound memory impairment (anterograde amnesia) 18 months after the poisoning (51–53). All the afflicted individuals had eaten mussels from Prince Edward Island near Newfoundland. Analysis of these mussels revealed high concentrations of domoic acid, an excitotoxic analog of glutamate that interacts selectively and powerfully with the KA receptor. A disproportionately high percentage of severely affected individuals were elderly. This provides an instructive example of an acute excitotoxic neurodegenerative process being induced in human adults by accidental exposure to a food-borne excitotoxic analog of glutamate. The apparent heightened vulnerability of elderly individuals to the neuropsychological consequences of domoate poisoning is of particular interest as this suggests that the KA receptor is a potentially sensitive mediator of excitotoxic neuropathology in old age. In Figure 1, note the structural similarities between the glutamic, kainic and domoic acid molecules.

Metabolic Disorders

SULFITE OXIDASE DEFICIENCY The first human neurodegenerative condition to receive specific attention as a possible excitotoxin-mediated phenomenon was sulfite oxidase deficiency (54), a rare inherited disease in which an abnormal metabolite, cysteine-S-sulfate accumulates in body tissues and disseminated degeneration of CNS neurons occurs resulting in blindness, spastic quadriplegia, and death in early infancy (55). It seems likely that cysteine-S-sulfate, acting by an excitotoxic mechanism, is responsible for neuronal degeneration in sulfite oxidase deficiency since cysteine-S-sulfate displays powerful excitotoxic activity when administered systemically to infant rats or microinjected into the adult rat brain (54).

OLIVOPONTOCEREBELLAR DEGENERATION It has been shown (56–58) that patients with the adult onset neurodegenerative disease, olivopontocerebellar degeneration (OPCD), have a deficiency of glutamic dehydrogenase enzyme activity that impairs their ability to metabolize Glu. Ingestion of Glu by such patients causes abnormally high levels of the amino acid in blood. Based on these findings, Plaitakis et al (57) have postulated a defect in CNS catabolism of Glu that may cause an excitotoxic build-up of Glu at EAA receptors with consequent slow degeneration of CNS neurons.

AMYOTROPHIC LATERAL SCLEROSIS Although individuals with amyotrophic lateral sclerosis (ALS) do not have a demonstrable deficiency of glutamic dehydrogenase, Plaitakis & Caroscio (59) have shown that they resemble OPCD patients in developing abnormally high blood Glu levels following oral intake of Glu. Thus, a build-up of Glu at CNS synapses on the basis of an unknown metabolic defect has been postulated to account for the degeneration of motor neurons in this disease. It should be borne in mind that if an exogenous excitotoxin such as BMAA can cause ALS-like degeneration of motor neuronal systems, it is possible that exogenous as well as endogenous excitotoxins may contribute to the development of the more classical form of ALS seen in the United States, i.e. given a metabolic defect (which remains to be deciphered) that compromises EAA metabolism in ALS, either exogenous or endogenous EAA may contribute to an excitotoxic build-up of EAA at CNS synapses and hence to motor system disease. Similar reasoning, of course, can also be entertained for OPCD.

Epilepsy, Hypoglycemia and Hypoxia/Ischemia

It has long been suspected that a common mechanism may underlie brain damage associated with prolonged seizures, hypoglycemia and hypoxia/ischemia because the histopathology in each case has similar characteristics. Energy deficiency has been proposed as a common denominator; however, studies have failed to demonstrate an energy deficit at the site of injury in epilepsy-related brain damage (60). Recent evidence implicating endogenous excitotoxins in the pathophysiology of each condition provides a common denominator that may help clarify the situation.

EPILEPSY Sustained limbic seizures induced in experimental animals by any of a number of methods results in brain damage which resembles Glu cytopathology and distributes in a pattern similar to that seen at autopsy in patients with intractable epilepsy (for a review of seizure-related brain damage, see 61). When sustained seizure activity is induced in specific pathways believed to use Glu as transmitter, it results in acute Glu-like cytopathology in dendrosomal neural elements postsynaptic to such pathways. It is possible to

prevent seizure-related brain damage by systemic administration of noncompetitive NMDA antagonists, including MK-801, PCP, and ketamine (62, 63). Curiously, however, the seizure activity, as recorded from depth electrodes in vulnerable brain regions, is not substantially suppressed by these agents. Thus, we tentatively propose that these agents block only one component of seizure activity (the component mediated through NMDA receptors that we postulate is the component responsible for seizure-related brain damage), while permitting seizure activity mediated through other pathways to continue relatively unabated.

HYPOGLYCEMIA In the human, severe hypoglycemia results in brain damage and in the rat hypoglycemic brain damage can be prevented by transection of glutamergic inputs to vulnerable regions (64) or by administration of NMDA antagonists (65). Moreover, Auer et al (66) have shown that hypoglycemic brain damage in the rat is indistinguishable, by either light or electronmicroscopic pathomorphological criteria, from Glu-induced brain damage.

HYPOXIA/ISCHEMIA In 1984, Rothman (17) demonstrated in an in vitro hippocampal cell culture preparation that EAA antagonists can prevent anoxic neuronal degeneration and Simon et al (67) showed that injection of a competitive NMDA antagonist into the hippocampus protects CA-1 hippocampal neurons from degenerating in an adult rat model of cerebral ischemia. In the same year, Benveniste et al (68) demonstrated that cerebral ischemia causes a marked elevation in the extracellular concentration of the endogenous excitotoxins, Glu and aspartate, in the rat hippocampus. Rothman's findings were readily confirmed in vitro (28, 69) and considerable evidence suggesting that NMDA antagonists can protect against hypoxic/ischemic brain damage in vivo has now been generated (70–76). Studies from the author's laboratory addressing this issue are summarized in Figures 3 and 4, the latter showing that hypoxic/ischemic and Glu-induced brain damage are indistinguishable by pathomorphological criteria.

CNS Trauma

BRAIN AND SPINAL CORD INJURY Katayama et al (77) recently demonstrated that concussive brain injury is associated with a fivefold increase in extracellular Glu concentrations in the rat hippocampus; this suggests that CNS tissue injury may entail an outpouring of endogenous excitotoxins from the intra- to extracellular compartment much as occurs under anoxic/ischemic conditions (68). Thus, edematous swelling or other brain tissue pathology associated with trauma may be due, at least in part, to an excitotoxic action of

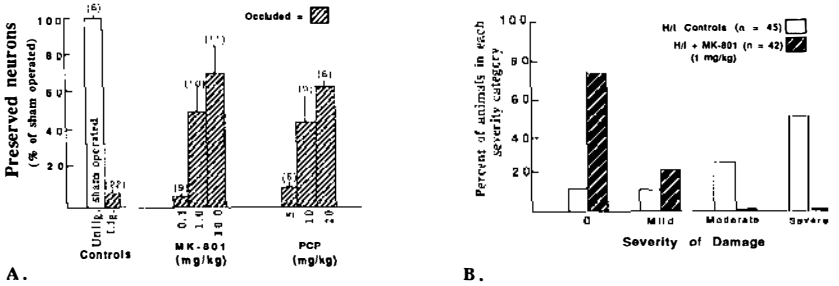

Figure 3 A. Adult gerbils were sham operated or subjected to bilateral carotid occlusion for 7.5 min and sacrificed 4 days later for histological evaluation. Some animals received MK-801 or PCP (ip) 15 min prior to carotid occlusion. Preserved CA 1 hippocampal neurons (per mm running row of CA 1 pyramids) were counted and plotted in terms of % of sham controls (73). B. Ten-day-old rats were subjected to hypobaric/ischemic (H/I) conditions (unilateral carotid ligation followed by 75 min in a hypobaric chamber at 225 mm Hg) and sacrificed 2 hr later for histological evaluation. Experimentals received MK-801 (1 mg/kg sc) 15 min prior to hypobaric exposure. The majority of H/I controls sustained relatively severe brain damage whereas the majority of MK-801 treated had no damage (74).

endogenous EAA. Consistent with this interpretation, it was recently reported that behavioral morbidity associated with head trauma (78) or spinal cord injury (79) is reduced by timely treatment with NMDA antagonists such as PCP and MK-801.

DEMENTIA PUGILISTICA Dementia pugilistica is a dementing illness associated with boxing (80). If concussive brain injury is associated with elevated extracellular hippocampal concentrations of endogenous excitotoxins (77), it is reasonable to propose that concussive blows delivered to the head in a boxing contest may cause a similar intra- to extracellular translocation of excitotoxins. It is conceivable that the initial concussive injury and associated excitotoxin outpourings may sensitize hippocampal or other CNS neurons to an excitotoxic process rendering them hypervulnerable to eventual degeneration as subsequent concussive blows re-expose EAA receptors to abnormal concentrations of endogenous excitotoxins. It is noteworthy that neurofibrillary tangles (aggregates of paired helical filaments), which are prominently present in the brain in Alzheimer's disease, are found in great abundance in the brains of dementia pugilistica victims (80). This establishes an interesting association between neurofibrillary tangles and repetitive exposure of EAA receptors to abnormal concentrations of endogenous excitotoxins. Reinforcing this association is the finding of DeBoni & McLachlan (81) that cultured human fetal spinal neurons develop paired helical filaments of the Alzheimer type when exposed to Glu or aspartate.

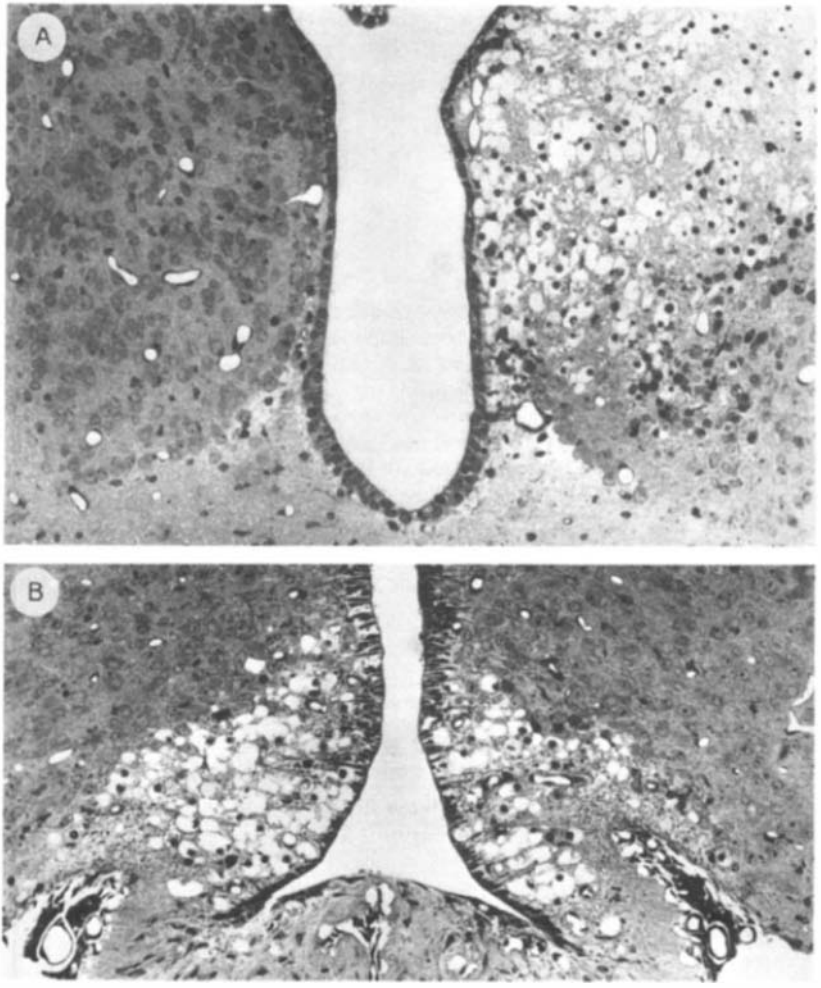

Figure 4 A. The medial habenular nucleus of a 10-day-old rat subjected to unilateral carotid ligation and 75 min in a hypobaric chamber followed by 4 hr recovery. The medial habenulum, a bilateral nucleus, exhibits damage unilaterally on the side ipsilateral to the carotid ligation. B. The arcuate hypothalamic nucleus of a 21-day-old mouse which had ingested a solution containing 10% monosodium glutamate 4 hr previously. Arcuate neurons are destroyed in a bilaterally symmetrical pattern. Note that the hypobaric/ischemic cytopathology (Figure 4A) appears identical to that induced by exogenous glutamate (Figure 4B). Degenerating neurons in each case typically have swollen edematous cytoplasm and dark pyknotic nuclei. In this hypobaric/ischemic model neurons undergo this type of acute degeneration in numerous brain regions, including the dentate hippocampal gyrus, caudate nucleus, frontoparietal neocortex, olfactory tubercle, dorsal subiculum, islands of calleja, and several thalamic nuclei (Mag = 200x) (adapted from Ikonomidou et al (108)).

Other

HUNTINGTON'S DISEASE The demonstration that injection of excitotoxins, such as KA and ibotenic acid, into the rat striatum results in biochemical and pathomorphological changes resembling those in Huntington's disease (82), provided a useful animal model for studying this neurodegenerative disease. More recent evidence that quinolinic acid, an excitotoxin found naturally in the brain, is more potent in destroying striatal than other CNS neurons (83, 84) but selectively spares a population of aspiny striatal neurons (85) that are also spared in Huntington's disease (86), has raised hopes that the pathophysiology of neuronal degeneration in Huntington's disease might be traced to quinolinic acid or a similarly selective endogenous excitotoxin. A role for quinolinic acid itself is questionable in view of recent evidence that quinolinic acid concentrations in the striatum of individuals with Huntington's disease is not elevated (87). However, this does not rule out the involvement of some other excitotoxic molecule with selective properties similar to those of quinolinic acid. It is thought that quinolinic acid acts predominantly at NMDA receptors and it has recently been shown that receptor loss in the striatum in Huntington's disease is disproportionately greater for NMDA than any other type of transmitter receptor (88).

ALZHEIMER'S DISEASE Several types of neurons, including cholinergic, somatostatinergic and noradrenergic, degenerate in Alzheimer's disease (AD). Loss of basal forebrain cholinergic neurons that project to the cerebral cortex, hippocampus and amygdala is a striking feature of the neuropathology of AD; it can be reproduced in experimental animals by injecting an excitotoxin into the basal forebrain region where these cells are located (89). This has provided a useful animal model for studying the role of cholinergic neurons in the cognitive deficits associated with AD. The fact that excitotoxins are effective in destroying cholinergic neurons implies that these neurons have EAA receptors through which endogenous excitotoxins could act pathologically to destroy these neurons. Alternatively, topical application of an excitotoxin to the cerebral cortex causes retrograde degeneration of those basal forebrain cholinergic neurons that project to the cortex (90). Thus, an excitotoxic process can cause neuronal degeneration by either a direct or indirect mechanism; since EAA receptors are present on many types of CNS neurons, an excitotoxic process, either direct or indirect, could explain the death of somatostatinergic and noradrenergic as well as cholinergic neurons. Arendash et al (91) recently described late-occurring pathological changes resembling neuritic plaques and neurofibrillary tangles in various limbic and neocortical regions of rat brain 14 months after injection of an excitotoxin into the basal forebrain to destroy cholinergic neurons. If this observation can be

corroborated, it becomes a tenable hypothesis that not only can the primary degeneration of various types of neurons in AD be explained by an excitotoxic process, but that other aspects of AD neuropathology (e.g. plaques and tangles) may arise as a delayed manifestation of or secondary reaction to this primary neurodegenerative process. Reinforcing this line of conjecture is the finding of DeBoni & McLachlan (81) that exposure of cultured human spinal neurons to abnormal concentrations of Glu or aspartate causes these neurons to produce paired helical filaments of the type that make up neurofibrillary tangles in AD. In addition, Procter and colleagues (92) have presented evidence for a possible metabolic defect affecting glutamergic neurons and Greenamyre et al (93) have described a striking loss of Glu receptors in the cerebral cortex and hippocampus of AD brains; however, the latter finding has been disputed (94). In summary, several lines of indirect evidence suggest that an excitotoxic mechanism could be involved in the pathophysiology of AD, but there is not enough direct evidence to establish the connection.

PARKINSONISM Although previous studies have not linked an excitotoxic mechanism with neuronal degeneration in Parkinsonism, Sonsalla et al (95) recently demonstrated that the neurotoxic action of methamphetamine against dopaminergic nigrostriatal neurons (a dopamine-dependent form of neurotoxicity) is blocked by MK-801. These authors suggest that methamphetamine neurotoxicity may resemble ischemic brain damage in the sense that oxidative stress and the unleashing of excitotoxic mechanisms may occur in both conditions. Since the neurons that degenerate selectively in Parkinsonism are dopaminergic nigrostriatal neurons, the demonstration that these neurons are vulnerable to excitotoxic injury triggered by a poorly understood dopamine-linked mechanism provides a basis for postulating that an excitotoxic mechanism could play a role in Parkinsonian neuronal degeneration. Also of interest is the observation (80) that parkinsonian symptoms and spontaneous degeneration of nigrostriatal neurons are features of the neuropathology in dementia pugilistica. Thus, it is possible that nigrostriatal neurons are sensitive to a pathological sequence in which either physical trauma or some toxic agent induces oxidative stress that unleashes the excitotoxic potential of endogenous EAA against these dopaminergic cells.

WERNICKE/KORSAKOFF SYNDROME In both alcoholism and nutritional deficiency syndromes, especially thiamine deficiency, neuronal degeneration has been described in several regions of the brain, including periventricular and periaqueductal regions, the thalamus, hypothalamus, and mammillary bodies. Individuals with this type of brain damage manifest the clinical syndrome known as Wernicke/Korsakoff syndrome, which includes severe

deficits in memory and cognitive functions. Langlais et al (96) recently demonstrated that in a rat model of thiamine deficiency, which entails disseminated brain damage distributed in a Wernicke/Korsakoff pattern, the brain damage can be markedly attenuated by pretreatment with MK-801. Thus, it is possible that NMDA receptors may play a role in the mediation of brain damage associated with alcoholism and nutritional deficiency syndromes.

JAKOB-CREUTZFELDT SYNDROME In this disease, which is known to have a viral etiology, neurons undergo a degenerative reaction in which the dendrites and cell bodies become grossly edematous and swollen, the plasma membrane ruptures and the nucleus displays pyknotic changes (97, 98). Since these are precisely the classical signs of Glu-induced neuronal degeneration (10), it is an intriguing possibility that viruses can induce changes in neuronal homeostasis that permit an unleashing of the excitotoxic potential of Glu and aspartate. In other words, endogenous excitotoxins may mediate the cell death process even though the etiologic agent is a slow virus. If a combined viral/excitotoxic mechanism does underlie neuronal degeneration in Jakob-Creutzfeldt disease, it is conceivable that a similar combination underlies other neurodegenerative diseases, including Alzheimer's disease.

NMDA RECEPTORS AND THE DEVELOPING CNS

Neurotrophism

The ubiquitous distribution of EAA transmitter networks throughout the CNS and their involvement in many important functions and systems (motor, sensory, autonomic, associational/cognitive, memory, etc) makes them logical candidates for complicity in a wide variety of neuropsychiatric disorders. Add to this the intrinsic excitotoxic potential of EAA systems and they become not only logical but prime candidates for such complicity. However, in addition to the toxic potential built into EAA systems, one of these—the NMDA system—has recently been shown to have a neurotrophic function that may play a vital role in the normal growth and development of the CNS (99–101). Thus, EAA systems literally have the power to promote the normal development of, or to destroy, many neurons in the mammalian CNS. Although excitotoxicity is the most obvious mechanism by which they may destroy neurons, discontinuation of neurotrophic support must be recognized as another potential means of effecting neuronal death. Moreover, a subtle formula for the genesis of neuropsychiatric disorders would be an interference in the neurotrophic functions of EAA systems causing the establishment of faulty connections during critical stages of CNS development.

Necrobiosis

As mentioned above, endogenous EAA may exert a neurotrophic action indispensable to the developing neuron while it is establishing its functional connections with other neural CNS components. A process of necrobiosis is recognized whereby neurons that fail to establish appropriate connections (so-called "redundant" neurons) are destroyed in the course of ontogenesis. It is possible that necrobiosis may be mediated by the simple termination of the neurotrophic influence of endogenous EAA at a time when a neuron that has not achieved its integrated status still needs neurotrophic assistance to survive. On the other hand, when neurons acquire the ability to excite other neurons by release of endogenous EAA, they presumably also acquire neurodestructive potential which must be held in check by various accessory mechanisms, such as EAA re-uptake transport processes and activation of Zn^{++}, Mg^{++} or PCP receptors. If substantial release of neuroexcitant should occur at NMDA receptors before these accessory mechanisms are in place to provide a counterbalancing action, excitotoxic destruction of the stimulated cell might occur. Conceivably, this may be a mechanism built into the CNS for eliminating redundant neural units. Although this is assumed to be a benevolent process whereby only redundant components are eliminated, the process might possibly become aberrant and destroy many additional neurons that are not redundant; indeed, how do we know that any neurons are truly redundant?

A Spectrum of Neuropsychiatric Disorders

In general, one can postulate either hyper- or hypofunction of EAA transmitter systems as the basis for neuropsychiatric disorders. As Figure 2 illustrates, normal function of the NMDA receptor depends on a dynamic equilibrium among multiple facilitative and inhibitory factors. Pathological processes might create an imbalance rendering the system either hypo- or hyperfunctional. For example, reduced efficacy of Glu or glycine or increased efficacy of Zn^{++}, Mg^{++}, or the endogenous PCP receptor ligand at their respective recognition sites would render the NMDA receptor system hypofunctional, and a mechanism affecting these factors in the converse direction would render the system hyperfunctional. Thus far, the major focus in excitotoxicology research has been on the hyperfunction concept—excessive activation of postsynaptic receptors can cause brain damage. However, when endogenous EAA transmitters, due to a hyperfunctional state, induce brain damage, it is essentially an "auto-excitotoxic" process in which the EAA neural network damages itself, i.e. the cells bearing EAA receptors are destroyed, as are the EAA receptors themselves, and the EAA neural system is rendered hypofunctional. In sum, regardless of whether the initial aberration is in the

hyper- or hypofunctional direction, the eventual result is likely to be a deficiency in EAA neurotransmission.

Subtle brain damage occurring in early stages of development has long been proposed to explain aberrant thought processes and psychotic symptoms in schizophrenia (102–105). It has also been proposed that hypofunction of the Glu transmitter system may underlie schizophrenia (106) based on the finding of reduced cerebrospinal fluid levels of Glu in schizophrenia. Although reproducibility of this finding has been challenged (107), recent evidence that the psychotomimetic agent, phencyclidine (PCP), powerfully inhibits NMDA receptor function (i.e. induces Glu transmitter hypofunction) strengthens the postulated connection between hypofunction of the Glu transmitter system and psychotic processes. One mechanism by which Glu hypofunction might occur would be by an auto-excitotoxic process, as mentioned above, in which subtle brain damage induced by an excitotoxic mechanism in early life would destroy postsynaptic cells that house the Glu receptor system, thereby rendering the Glu neural network deficient in excitatory receptors. Thus, a unifying excitotoxic hypothesis could accommodate both concepts (early brain damage and Glu hypofunction) to explain the symptomatology of schizophrenia.

It is difficult to predict what type of symptoms would be produced by Glu hypofunction. It would entail a loss of neurons that are normally excited by Glu; however, this would include inhibitory neurons such as GABAergic neurons, and loss of inhibitory neurons from a given pathway would disinhibit that pathway making it hyperreactive to normal levels of stimulation. It has often been proposed that attention deficit disorder (hyperkinetic child syndrome) might be explained by subtle brain injury during development. It is quite conceivable that an auto-excitotoxic process may cause a type of brain injury in fetal life that could give rise to hyperkinetic behavior in childhood.

Recently we have been exploring a new model for studying hypoxic/ischemic brain damage in which infant rats are subjected to unilateral carotid ligation followed by 75 minutes in a hypobaric chamber (225 mm Hg). We have shown (108) that this approach results in acute neuronal necrosis disseminated over many brain regions (frontoparietal neocortex, caudate/putamen, thalamus, hippocampus, medial habenulum, septum, and olfactory tubercle) and that the acute neurodegenerative reaction is identical, both in time course and type of cytopathology, to that observed in the hypothalamus of immature rodents (10) or monkeys (8) treated systemically with glutamate (Figure 4). We have also corroborated the observation of McDonald et al (109) that the infant rat is much more sensitive than the adult rat to the neurotoxic action of NMDA (110). In contrast, the infant rat brain is less sensitive than the adult to kainic acid neurotoxicity (111). Injection of

nanomolar amounts of NMDA directly into the infant rat brain causes widespread neuronal degeneration that appears identical to hypobaric/ischemic neuronal degeneration, and the neuronal populations most sensitive to NMDA toxicity are the same as those most sensitive to hypobaric/ischemic degeneration (110). Moreover, administration of the NMDA-specific antagonist, MK-801, provides excellent protection against either NMDA neurotoxicity or hypobaric/ischemic neuronal degeneration (74, 112). These findings strongly implicate the NMDA receptor in perinatal hypoxic/ischemic brain damage.

Extending the above comparison further, we have found that sensitivity to either NMDA neurotoxicity or to hypobaric/ischemic brain damage increases during the first few days of life to reach peak sensitivity in the rat between the 6–10th postnatal days and steadily declines for several weeks thereafter (110). This suggests that there is a period spanning approximately the first two weeks of neonatal life in the rat during which NMDA receptors may be hypersensitive to stimulation. We propose that during this period, CNS neurons bearing such receptors may be hypervulnerable to excitotoxic degeneration, i.e. only mild anoxia or oxidative stress may be sufficient to trigger neuronal degeneration. We also have observed that each neuronal group is governed by its own timetable for onset and duration of the period of peak sensitivity (110). If the developing human is subject to a similar phenomenon, we propose that the period of NMDA receptor hypersensitivity in the human may span months rather than weeks and that different combinations of CNS neurons may be hypervulnerable at any given time during this period. Thus, a pathological process involving NMDA receptors might produce several different patterns of neuronal loss depending on the developmental stage in which the pathological event occurred. If so, any of several neuropsychiatric deficit syndromes (e.g. cerebral palsy, hyperkinesis, childhood autism, or schizophrenia) may result in later life.

An additional consideration is the possibility that without prior brain damage, NMDA receptor dysfunction may arise de novo in young adult life to give rise to schizophrenic symptomatology. Again, in view of the complicated makeup of the NMDA receptor-ionophore complex, there are a number of possible mechanisms by which hypofunction of this complex may take place, hence a number of possible mechanisms by which psychotic symptoms may be induced.

NMDA RECEPTORS AND MEMORY

Given recent evidence (113) for involvement of the NMDA receptor in long-term potentiation (LTP) and the putatively related process, memory formation, it is of interest to consider possible mechanisms by which dysfunction of the NMDA receptor system may give rise to memory impairment. The

most obvious mechanism would be a process whereby cells bearing NMDA receptors are destroyed as a result of a neurodegenerative process, i.e. if cells possessing NMDA receptors are destroyed, the NMDA receptors would also be destroyed and thereby eliminated from further participation in memory functions. However, given the complex makeup of the NMDA receptor-ionophore system, there are several mechanisms by which this system could be rendered dysfunctional without actual destruction of either the receptor complex or the host cell; for example, any mechanism leading to excessive activation of Zn^{++}, Mg^{++}, or PCP membrane sites or decreased activation of NMDA or Gly receptors may render the complex hypofunctional and result in memory impairment. Consistent with this hypothesis, Morris et al (114) reported that intracerebroventricular injection of the NMDA antagonist, D-AP5, blocks the in vivo development of LTP and interferes with spatial learning. Moreover, in 1983, before the relationship between PCP and NMDA receptors was fully appreciated, Stringer and colleagues (115) demonstrated that PCP and related compounds block LTP in both the in vitro and in vivo hippocampus. Other recent evidence suggests that either PCP (116) or MK-801 (117–118), when systemically administered to adult rats, interferes with memory formation.

NMDA RECEPTORS AND AGING

In mid-adult life, it is believed the CNS neurons begin to die and that there is a steady attrition of neurons from that point unto death. Considering the several agonist and antagonist principles that constitute counterbalancing components of the NMDA/Gly/PCP/Zn^{++}/Mg^{++} receptor-ionophore complex, and evidence that this receptor-ionophore complex is widely distributed throughout the forebrain, it is not unreasonable to postulate an age-linked shift in the dynamic equilibrium of this system such that the agonist driving mechanisms overwhelm the antagonist forces in the microenvironment of a given neuron, the result being excitotoxic death of that neuron. Such a process could account for the "normal" death of a large number of CNS neurons as a function of age.

Recent evidence (51) that domoate poisoning results in particularly severe neuropsychological deficits in elderly individuals suggests that KA receptors may be sensitive mediators of excitotoxicity in old age. However, based on observations pertaining to KA toxicity, brain damage associated with domoate poisoning may be seizure-mediated, in which case although the seizure activity is initiated by activation of KA receptors, seizure-mediated release of Glu at NMDA receptors may be responsible for much of the brain damage (62, 63). Therefore, it seems likely that in the elderly human both KA and NMDA receptors may be sensitive mediators of excitotoxicity.

CONCLUSIONS

Here I have discussed current issues in excitotoxicology research with special emphasis on the NMDA receptor and its possible role in neurodegenerative diseases. I have briefly described several classes of anti-excitotoxic agents currently under study for their ability to protect neurons against excitotoxin-mediated neuronal degeneration. There is growing interest in the possibility that such agents, especially NMDA antagonists, will prove useful in the clinical management of neurodegenerative disorders; however, neither their efficacy nor safety has been adequately established at present. With the plethora of new information about the NMDA receptor-ionophore complex, one tends to forget that non-NMDA receptors can also mediate excitotoxic events. An instructive case in point is the recent evidence implicating KA receptors in domoate poisoning in which the resultant dementia is manifested most prominently in the elderly. Thus, although we know less about the physiology and makeup of non-NMDA receptors, as new information becomes available, it will probably lead to the recognition of new links between both NMDA and non-NMDA receptor-mediated processes and neuropsychiatric disorders. It is wise, therefore, to keep an open mind regarding the ultimate significance that can be ascribed to excitotoxic processes in human neuropsychiatric diseases, and the promise of anti-excitotoxic strategies for preventing such diseases.

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